New QuikChange protocol

QuikChange is a protocol to quickly insert a single bp mutation, or a codon insertion or deletion into a plasmid.

1. Design primers
    a. Check sequence and determine mutation that will be made
    b. Make a forward and reverse primer that contains the mutation, but that do not completely overlap. This will help drive annealing to the plasmid and not to the other primer. Tm for each primer should be close to or over 70°C.

    Example of primer overlap

5’	G	A	G	C	T	G	T	C	A	T	C	G	T	G	C	3’					
					3’	C	A	G	T	A	G	C	A	C	G	T	A	G	T	G	C	5’

2. PCR
    a. Use platinum pfx DNA polymerase kit from Invitrogen
        i. Add to pcr mixture

        5 µl 10 x Pfx platinum DNAP buffer
        1 µl 50 mM $MgSO_4$
        2 µl 10 mM dNTP mix
        1.5 µl 10 µM forward primer
        1.5 µl 10 µM reverse primer
        20-80 ng Template DNA
        0.5 µl Pfx Platinum DNA polymerase
        Fill to 50 µl with DI water

        ii. Thermocycler program

        Step 1 5 min at 94°C
        Step 2 45 sec at 94°C
        Step 3 45 sec at 55°C
        Step 4 1 min/kb at 68°C
        Repeat steps 2-4 for 16-18 cycles
        Step 5 10 min at 68°C
        Hold at 4°C
3. Digest with 1 µl of DpnI RE for 1 hour at 37°C. Digestion can be done in the pcr buffer.
    a. Digesting with DpnI will digest any methylated DNA. This should eliminate any of the template DNA and leave only single stranded PCR product containing the desired mutation
4. Run pcr samples on agarose gel to check for amplification
5. Transform 1 µl of digested pcr product into electrocompetent cells (use own protocol here). This will be a low efficiency transformation so make sure to plate all of the cells to ensure colonies.
6. Grow and screen colonies.